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Biosynthetic Studies of Marine Lipids. 11.[1] Synthesis, Biosynthesis, and Absolute Configuration of the Internally Branched Demospongic Acid, 22-Methyl-5,9-octacosadienoic Acid

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The ^{14}C-labeled short-chain fatty acid 10-methylhexadecanoic acid (10-Me-16:0) as well as its hitherto undescribed 10*R* and 10*S* antipodes was incorporated into the marine sponge *Aplysina fistularis* and transformed in situ into 22-methyl-5,9-octacosadienoic acid (22-Me-$\Delta^{5,9}$-28:2) by chain elongation. No qualitative specificity between the two enantiomers was observed. [^3H]Methionine was also incorporated into the sponge to investigate the methylation process; a rapid incorporation into the short branched fatty acids occurred with subsequent chain elongation. Both enantiomers were synthesized starting from (+)-pulegone to determine the absolute configuration of the 22-methyl-5,9-octacosadienoic (22-Me-$\Delta^{5,9}$-28:2) acid. The naturally occurring acid has the 22*R* configuration.

Introduction

Extensive studies of the fatty acid composition of marine sponges showed that most of these animals contain large amounts of long-chain C_{24}–C_{30} fatty acids (LCFA's).[2-7] These acids may have straight or branched carbon skeletons, including terminal or internal methyl branching, as is seen in the sponges *Aplysina fistularis*,[5] *Stronglyophora durissima*,[6] and *Petrosia hebes*.[7]

To date very little is known about the biosynthesis of these acids. The single relevant publication by Morales and Litchfield[8] recorded the incorporation of labeled acetate into the sponge acids 5,9-hexacosadienoic and 5,9,19-hexacosatrienoic acids; based on this result, they proposed that these acids were generated by chain elongation with subsequent introduction of double bonds at C-9 and C-5. Recent work in our laboratory demonstrated that the terminally branched, 25-methyl-5,9-hexacosadienoic and 24-methyl-5,9-hexacosadienoic acids in the sponge *Jaspis stellifera* originate via chain elongation of short-chain iso or anteiso fatty acids.[9] Since such terminal methyl branching is typical of bacterial fatty acids,[10] this result suggests that the precursors are of bacterial origin with later homologation and double-bond introduction.

Because of the unusual branching in the central portion of the chain, we were especially interested in 22-methyl-5,9-octacosadienoic acid (22-Me-$\Delta^{5,9}$-28:2) (**1**) present in *A. fistularis*. The biosynthesis of this acid can be visualized to occur either by conventional chain elongation with subsequent methylation by *S*-adenosylmethionine of an olefinic intermediate such as 5,9,21-octacosatrienoic ($\Delta^{5,9,21}$-28:3) acid (which is known to occur in that sponge)[5] or via a branched bacterial precursor of shorter chain length that is then homologated and desaturated. To distinguish between these two possibilities, we incorporated the following ^{14}C-labeled short-chain precursors into *A. fistularis*: *dl*-10-methylhexadecanoic acid (10-Me-16:0) (**6**), palmitic acid (16:0), and palmitoleic acid (Δ^9-16:1) (**29**). We also synthesized in radioactive form the two enantiomers, (10*R*)-10-methylhexadecanoic ((10*R*)-10-Me-16:0) (**26**) and (10*S*)-10-methylhexadecanoic ((10*S*)-10-Me-16:0) (**27**) acids, to investigate the effect of chirality at a fairly distant center in the chain elongation process toward the long-chain demospongic acids.

We considered 10-methylhexadecanoic acid to be an appropriate precursor since it is present in the phospholipids of *A. fistularis*[5] and is also present in other sponges

(1) For Part 10 in this series, see: Stoilov, I.; Thompson, J. E.; Djerassi, C. *Tetrahedron Lett.* **1986**, *27*, 4821–4824.
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(3) Litchfield, C.; Tyszkiewicz, J.; Dato, V. *Lipids* **1980**, *15*, 200–202.
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(6) Dasgupta, A.; Ayanoglu, E.; Djerassi, C. *Lipids* **1984**, *19*, 768–776.
(7) Wijekoon, W. M. D.; Ayanoglu, E.; Djerassi, C. *Tetrahedron Lett.* **1984**, *25*, 3285–3288.
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(10) Nes, W. R.; Nes, W. D. *Lipids in Evolution*; Plenum Press: New York, 1980; pp 88–142.

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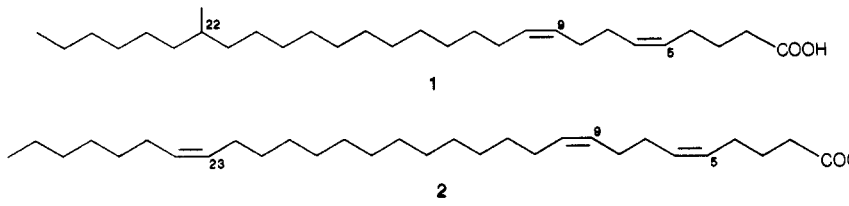

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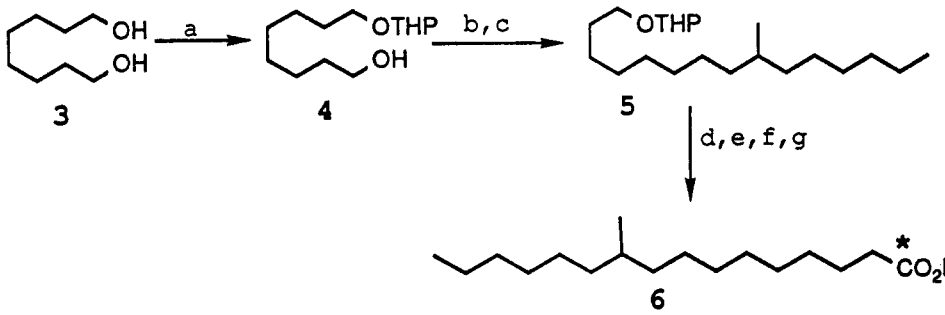
Scheme I[a]



3 4 5 6

[a] Reagent: (a) dihydropyran, H^+, Et_2O; (b) TsCl, pyridine; (c) Li_2CuCl_4, 2-octylmagnesium bromide; (d) TsOH, MeOH; (e) MsCl, Et_3N, DMAP, CH_2Cl_2; (f) $K^{14}CN$, THF, Me_2SO; (g) KOH, EtOH.

at low concentrations.[11] Short-chain fatty acids with internal branching are widely distributed in microorganisms, particularly in bacteria.[12,13] Generally, internally branched acids are thought to be of microbial origin.[14] Their biosynthesis has been thoroughly studied in bacteria;[15,16] they are derived from the corresponding olefinic acids by methylation of the double bond via *S*-adenosylmethionine. However, 10-methylhexadecanoic acid (**6**) seems to be relatively rare[17] in nature; it has been found in relatively high concentrations in marine sulfate-reducing bacteria.[18,19]

Results and Discussion

The detailed fatty acid composition of *A. fistularis* was studied earlier in our laboratory.[5] The total phospholipid fatty acids contain large amounts of short-chain fatty acids that are assumed to be primarily of bacterial origin[9] and two unusual long-chain fatty acids (**1**, **2**) that are almost certainly sponge metabolites.[5] The major phospholipid fatty acids are given in Table I. Their relative abundances vary over time.

[1-^{14}C]-*dl*-10-Methylhexadecanoic (**6**), [1-^{14}C]-(10*R*)-10-methylhexadecanoic (**26**), [1-^{14}C]-(10*S*)-10-methylhexadecanoic (**27**), and [1-^{14}C]palmitoleic (**29**) acids were synthesized as described in Schemes I–IV to perform the biosynthetic experiments. The [1-^{14}C]-*dl*-10-methylhexadecanoic acid (**6**) was prepared (Scheme I) by tosylation of 1,8-octanediol monotetrahydropyranyl ether (**4**), coupling with 2-octylmagnesium bromide in the presence of dilithium tetrachlorocuprate,[20] conversion of the tetrahydropyranyl ether **5** to the alcohol, mesylation, treatment with 1 mCi (57.6 mCi/mmol) of $K^{14}CN$ to afford the corresponding nitrile, and final hydrolysis to the labeled 10-methylhexadecanoic acid (**6**).

Table I.[a] **Major Fatty Acids from the Phospholipids of *Aplysina fistularis***

compd	ECL[b]	fatty acid	abundance[c]
1	14.63	13-methyltetradecanoic (iso-15:0)	10.2
2	14.72	12-methyltetradecanoic (antieiso-15:0)	8.5
3	15.80	9-hexadecenoic (Δ^9-16:1; palmitoleic)	4.7
4	16.42	15-methyl-9-hexadecenoic (Δ^9-iso-17:1)	5.8
5	16.43	10-methylhexadecanoic (10-Me-16:0)	4.2
6	17.86	11-octadecenoic (Δ^{11}-18:1)	7.1
7	18.40	11-methyloctadecanoic (11-Me-18:0)	5.3
8	28.12	22-methyl-5,9-octacosadienoic (22-Me-$\Delta^{5,9}$-28:2)	4.6
9	29.62	5,9,23-triacontatrienoic ($\Delta^{5,9,23}$-30:3)	4.1

[a] Capillary gas chromatography on fused silica column SE54 (30 m × 0.32 mm, J&W Scientific, Inc.); program temperature, 130–290 °C, 5°/min. [b] Equivalent chain length values are those of the methyl esters of the acids. [c] Average composition of samples collected over 1 year.

Since the chirality of the natural 22-methyl-5,9-octacosadienoic acid (**1**) is not known, we also synthesized the two optical antipodes of the 10-methylhexadecanoic acids (Schemes II and III) for biosynthetic experiments. The chiral precursors were prepared starting from (*R*)-(+)-pulegone (**7**), which was transformed to (*R*)-(+)-citronellic acid (**8**) and (*R*)-(+)-citronellol (**9**) according to the literature procedure.[21] The optical purity of **8** was determined by its derivatization to the amide **13** with (*S*)-(−)-α-methylbenzylamine.[22] Analysis of the amide by normal-phase HPLC gave a separation of the diastereomers and indicated that the enantiomeric purity of the acid **8** is 97%, assuming that the (*S*)-(−)-α-methylbenzylamine used was 100% optically pure.[23] Citronellol (**9**) was then

(11) Gillan, F. T.; Thompson, J. E.; Hogg, R. W.; Stoilov, I.; Djerassi, C., in preparation.
(12) Campbell, I. M.; Naworal, J. *J. Lipid Res.* **1969**, *10*, 593–598.
(13) Hofheinz, W.; Grisebach, H. Z. *Naturforsch. B*: **1965**, *20B*, 43–53.
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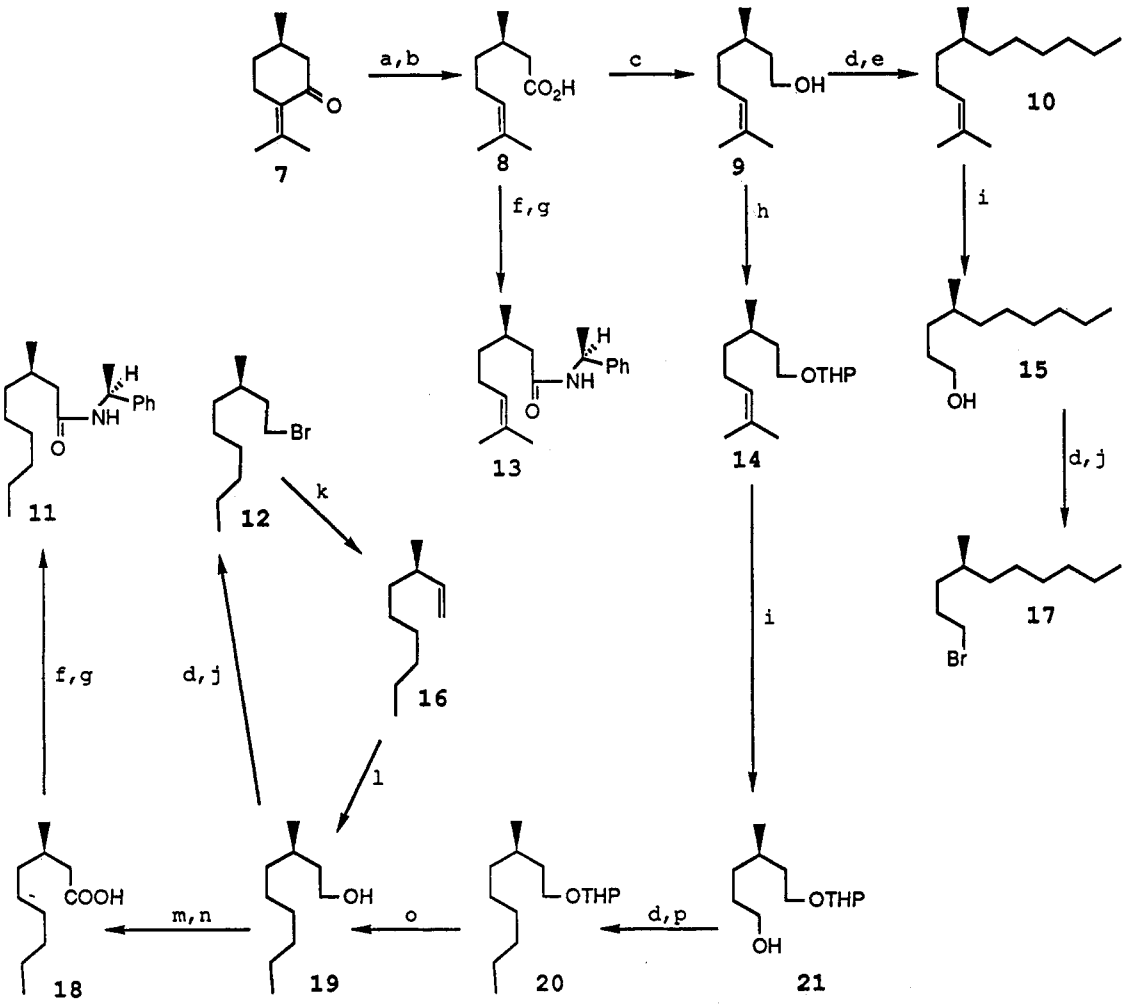
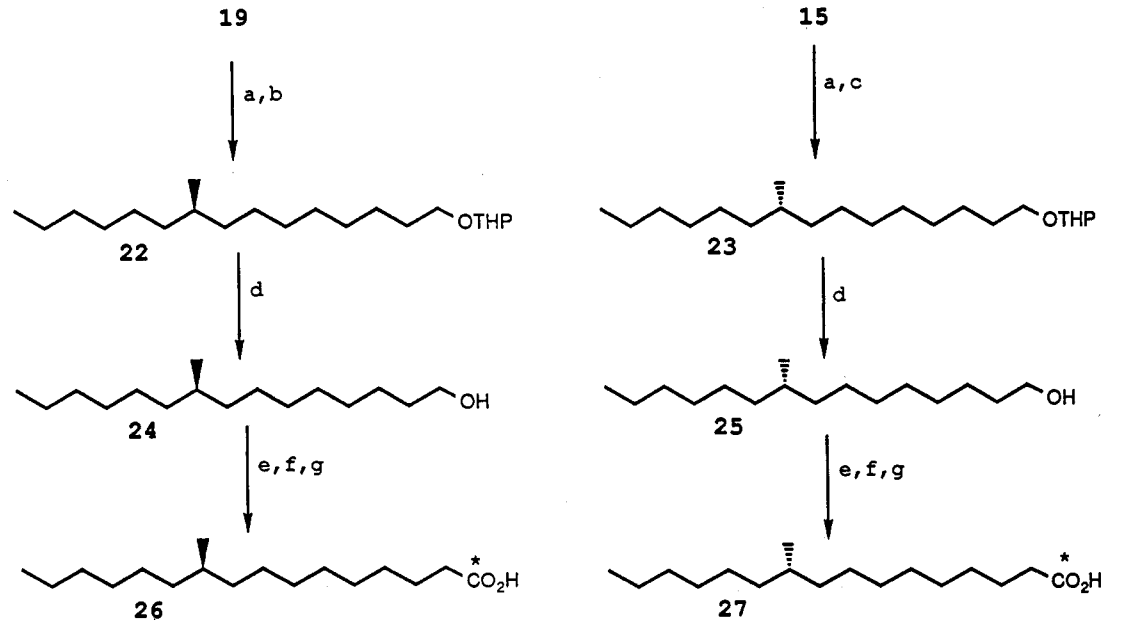
(17) Volkman, J. K.; Johns, R. B.; Gillan, F. T.; Perry, G. J.; Bavor, H. J. *Geochim. Cosmochim. Acta* **1980**, *44*, 1133–1143.
(18) Taylor, J.; Parkes, R. J. *J. Gen. Microbiol.* **1983**, *129*, 3303–3309.
(19) Current work in our laboratory has shown that such anaerobic sulfate-reducing bacteria are present in *A. fistularis*.
(20) (a) Fouquet, G.; Schlosser, M. *Angew. Chem., Int. Ed. Engl.* **1974**, *13*, 82–83. (b) Schlosser, M. *Ibid.* **1974**, *13*, 701–706.
(21) Plesck, J. *Collect. Czech. Chem. Commun.* **1957**, *22*, 644–646.
(22) Valentine, D.; Chan, K. K.; Scott, C. G.; Johnson, K. K.; Toth, K.; Saucy, G. *J. Org. Chem.* **1976**, *41*, 62–65.

Scheme II[a]



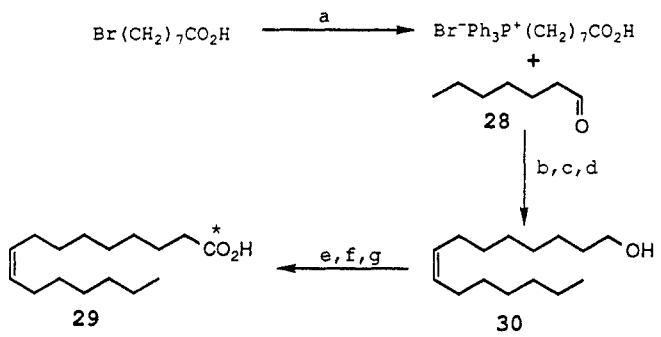
[a] Reagent: (a) HCl; (b) KOH; (c) $LiAlH_4$, Et_2O; (d) TsCl, pyridine; (e) Li_2CuCl_4, *n*-BuMgBr; (f) $(COCl)_2$, benzene; (g) (*S*)-(−)-α-methylbenzylamine, Et_2O; (h) dihydropyran, H^+; (i) O_3, $NaBH_4$; (j) LiBr, acetone; (k) PhSeSePh, $NaBH_4$, EtOH, H_2O_2; (l) 9-BBN, NaOH, H_2O_2; (m) PDC, CH_2Cl_2; (n) Ag_2O; (o) TsOH, MeOH; (p) Li_2CuCl_4, *n*-PrMgBr.

Scheme III[a]



[a] Reagent: (a) TsCl, pyridine; (b) Li_2CuCl_4, $THPO(CH_2)_6MgCl$; (c) Li_2CuCl_4, $THPO(CH_2)_5MgCl$; (d) TsOH, MeOH; (e) MsCl, Et_3N, DMAP, CH_2Cl_2; (f) $K^{14}CN$, THF, Me_2SO; (g) KOH, EtOH.

Scheme IV[a]



[a] Reagent: (a) Ph_3P, heat; (b) *n*-BuLi, THF, Me_2SO; (c) MeOH, H^+; (d) $LiAlH_4$, THF; (e) MsCl, Et_3N, DMAP, CH_2Cl_2; (f) $K^{14}CN$, THF, Me_2SO; (g) KOH, EtOH.

converted to the alkene **10** by tosylation and coupling with *n*-butylmagnesium bromide in the presence of a catalytic amount of dilithium tetrachlorocuprate.[20] Ozonolysis of the alkene **10** followed by reductive workup with $NaBH_4$ gave the alcohol **15**, which was tosylated and coupled with the Grignard reagent derived from 5-hydroxypentyl chloride tetrahydropyranyl ether to yield the ether **23** (Scheme III). Deprotection to the alcohol **25**, mesylation, one-carbon extension with $K^{14}CN$, and hydrolysis led to the desired [1-^{14}C]-(10*S*)-10-methylhexadecanoic acid (**27**).

The other enantiomer (**26**) was obtained by protection of the hydroxyl group of (*R*)-(+)-citronellol (**9**) though its tetrahydropyranyl ether **14**,[24] which was ozonized in the same manner as before to the alcohol **21**. Tosylation and a three-carbon homologation with *n*-propylmagnesium bromide furnished the tetrahydropyranyl ether **20**. Removal of the protective group[25] generated the alcohol **19**. Since the optical rotation of this alcohol **19** did not agree with that reported in the literature,[26] its optical purity was verified by oxidation of the alcohol **19** to its aldehyde with pyridinium dichromate,[27] followed by further oxidation with silver oxide to the acid **18**.[28] Subsequent derivatization with (*S*)-(−)-α-methylbenzylamine led to the amide **11**, which showed that the enantiomeric ratio of the alcohol **19** remained the same as that of its synthetic precursor (*R*)-(+)-citronellic acid (**8**).[29] The alcohol **19** was then tosylated and coupled (Scheme III) with the Grignard reagent prepared from the tetrahydropyranyl ether of 6-hydroxyhexyl chloride to give the tetrahydropyranyl ether **22**. The remaining steps to the [1-^{14}C]-(10*R*)-10-methylhexadecanoic acid (**26**) were the same as described for its enantiomer **27**.

The synthesis of the labeled palmitoleic acid (**29**) (Scheme IV) involved a Wittig reaction[30] of 1-heptaldehyde with (7-carboxyheptyl)triphenylphosphonium bromide to (*Z*)-8-pentadecenoic acid, which was reduced to the alcohol **30** and then transformed in the standard manner into the desired [1-^{14}C]-9-hexadecenoic acid (**29**).

The ^{14}C-labeled precursors were incorporated as their sodium salts into live, whole sponges in aquaria; the sponges were subsequently transferred to flowing-seawater aquaria for 1 month, since shorter periods of time gave inferior results (see Table II). The isolations and analyses of the different fractions were then carried out on the lyophilized sponge.

The radioactivity was determined for the phospholipid fraction after separation from neutral lipids and glycolipids. Some activity was found in the neutral lipids but the fatty acid analysis of this fraction showed that the LCFA's were present in lower proportions than in the phospholipids[31] and almost all the activity was associated with the short-chain fatty acids. Only traces of radioactivity were found in the glycolipid fraction. These results are in agreement with earlier studies that showed that the LCFA's are mainly in the phospholipid fraction[4] and are membrane components.[32]

The distribution of the radioactivity in the fatty acid methyl esters from the phospholipids of *A. fistularis* was assessed (Table II) after purification and separation into the individual components by HPLC (Table II). As predicted, the appropriate short-chain precursors are effectively converted into the branched or straight LCFA's, even

Table II. Distribution of Radioactivity (dpm) in the Phospholipid Fatty Acids of *Aplysina fistularis*

	^{14}C precursor[a]	total fatty acids recovered	22-Me-$\Delta^{5,9}$-28:2 (**1**)	$\Delta^{5,9,23}$-30:3 (**2**)
1.	*dl*-10-Me-16:0 (**6**) 20 μCi, 7 days	3.4×10^5	3360	<500
2.	*dl*-10-Me-16:0 (**6**) 20 μCi	3.6×10^5	11780	<500
3.	*dl*-10-Me-16:0 (**6**) 40 μCi	7.35×10^5	42890	<500
4.	(10*R*)-10-Me-16:0 (**26**) 20 μCi	4.38×10^5	20370	<500
5.	(10*R*)-10-Me-16:0 (**26**) 40 μCi	8.04×10^5	32420	<500
6.	(10*R*)-10-Me-16:0 (**26**) 40 μCi[b]	4.92×10^5	36190	<500
7.	(10*S*)-10-Me-16:0 (**27**) 20 μCi	6.12×10^5	7430	<500
8.	(10*S*)-10-Me-16:0 (**27**) 20 μCi	6.36×10^5	10500	<500
9.	(10*S*)-10-Me-16:0 (**27**) 40 μCi[b]	7.76×10^5	21140	<500
10.	*n*-16:0 20 μCi	1.49×10^5	<500	<500
11.	Δ^9-16:1[c] (**29**) 20 μCi	9.8×10^5	<500	7070

[a] Each precursor was incorporated in a separate experiment for 30 days if not specified. [b] Incorporation performed on the same sponge specimen cut in half. [c] In this case 99640 dpm was isolated in the 10-Me-16:0.

Table III. Distribution of Radioactivity (dpm) after Ozonolysis of the 22-Me-$\Delta^{5,9}$-28:2-acid (1)

^{14}C precursor	activity in the 22-Me-$\Delta^{5,9}$-28:2 (**1**)	activity in the fission compounds: 13-Me-19:0	activity in the fission compounds: C_4 and C_5 diacids
dl-10-Me-16:0 (**6**)	19450	16980	<200
(10*R*)-10-Me-16:0 (**26**)	19000	16880	<200
(10*S*)-10-Me-16:0 (**27**)	17350	16090	<200

(23) (*S*)-(−)-Citronellol (from Fluka) was oxidized to the acid and converted with the same (*S*)-(−)-α-methylbenzylamine to obtain the corresponding diastereoisomer.
(24) Bernady, K. F.; Floyd, M. B.; Poletto, J. F.; Weiss, M. *J. Org. Chem.* **1979**, *44*, 1438–1447.
(25) Corey, E. J.; Niwa, H.; Knolle, J. *J. Am. Chem. Soc.* **1978**, *100*, 1942–1943.
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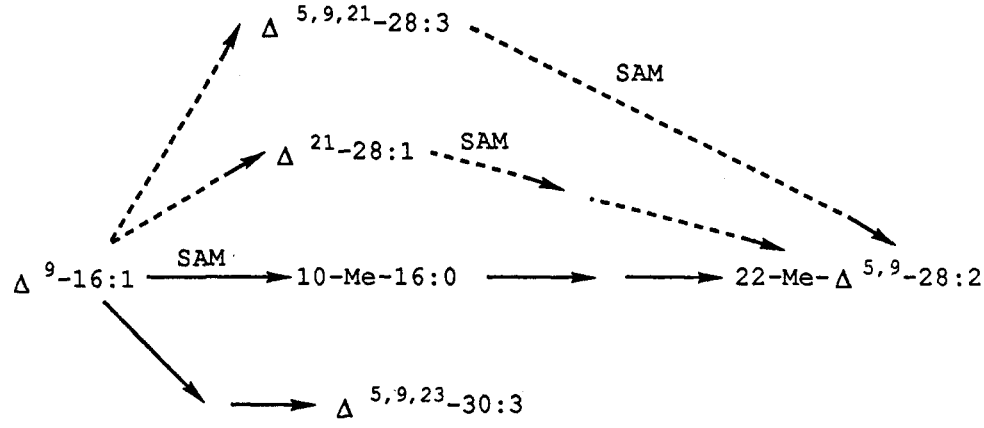
(27) Corey, E. J.; Schmidt, G. *Tetrahedron Lett.* **1979**, 399–402.
(28) Campaigne, E.; LeSuer, W. M. *Organic Syntheses*; Wiley: New York, 1963; Collect. Vol. 4, pp 919–921.
(29) The (*S*)-(−)-citronellol from Fluka (which was shown to have the enantiomeric ratio of (93:7))[23] was transformed in the same way to give the enantiomer of the alcohol **19**, which was converted with the same (*S*)-(−)-α-methylbenzylamine to the diastereomer of **11**. The HPLC analysis indicated that the enantiomeric ratio remained the same.
(30) Bestmann, H. J.; Vostrowsky, O.; Platz, H. *Chem. Ztg.* **1974**, *98*, 161–162.
(31) Lawson, M. P.; Thompson, J. E.; Djerassi, C., unpublished results.
(32) Lawson, M. P.; Bergquist, P. R.; Cambie, R. C. *Tissue Cell* **1986**, *18*, 19–26.

Scheme V.[a] **Biosynthetic Pathways to LCFA's in *A. fistularis***



[a] Hypothetical alternatives are indicated by dashed lines.

Table IV. Distribution of Radioactivity (dpm) in the Phospholipid Fatty Acids after Incubation of [^{3}H]Methionine (20 μCi)

time of incubation	total fatty acids recovered	10-Me-16:0	11-Me-18:0	22-Me-$\Delta^{5,9}$-28:2 (1)	$\Delta^{5,9,23}$-30:3 (2)
7 days	1.83×10^5	28700	22600	<100	<100
30 days	1.56×10^5	36700	14600	1830	<100

though a high amount of radioactivity remained in the precursors.

The 22-Me-$\Delta^{5,9}$-28:2 acid (**1**) was ozonized by using the BF_3/MeOH[33] workup procedure to further localize the radioactivity in the LCFA's, and the cleavage products were separated by HPLC. As expected, the radioactivity remained with the 13-methylnonadecanoic acid (13-Me-19:0) (Table III) and no significant activity was found in the C_4 and C_5 dicarboxylic acids. Some radioactivity was also lost during the reaction and purification; this result clearly shows that degradation by β-oxidation and resynthesis does not take place to a significant extent in the 1-month experiment. The $\Delta^{5,9,23}$-30:3 acid was also subjected to epoxidation with *m*-chloroperoxybenzoic acid; after purification by TLC all the radioactivity remained with the pure triepoxide, demonstrating that the radioactivity is associated with this compound.

To investigate the methylation process, we also administered [^{3}H]methionine to the sponge. The phospholipid fatty acid methyl esters were analyzed 7 and 30 days after incorporation of the precursor (Table IV). It appears that methionine is very rapidly incorporated into the branched short-chain fatty acids, but only very slowly into the branched LCFA's.

From the incorporation experiments four major conclusions were reached: (1) The labeled *dl*-10-Me-16:0 acid (**6**) was incorporated into the branched 22-Me-$\Delta^{5,9}$-28:2 acid (**1**) but not into the straight chain $\Delta^{5,9,23}$-30:3 acid (**2**). This provides further evidence that β-oxidation of the labeled precursor to acetate and resynthesis is of no concern in this instance. (2) When the optically active compounds were used, both (10*R*)- (**26**) and (10*S*)-10-Me-16:0 (**27**) precursors were converted into the 22-Me-$\Delta^{5,9}$-28:2 demospongic acid (**1**). Triplicate experiments (Table II) show that the incorporation of the (10*R*)-10-Me-16:0 (**26**) acid is somewhat more efficient than that of the 10*S* antipode (**27**). (3) When the *n*-16:0 acid (palmitic acid) was fed, no activity could be detected in either the 22-Me-$\Delta^{5,9}$-28:2 (**1**) or the $\Delta^{5,9,23}$-30:3 (**2**) acids. (4) Palmitoleic acid (Δ^9-16:1) (**29**) was incorporated into the $\Delta^{5,9,23}$-30:3 acid (**2**) but not into the branched 22-Me-$\Delta^{5,9}$-28:2 acid (**1**).

The different possible biosynthetic pathways are summarized in Scheme V. Judging from the methionine experiment (Table IV), it is not certain whether the methylation of a long-chain olefinic acid with a Δ^{21} double bond via *S*-adenosylmethionine is operative. The methylation did occur effectively with palmitoleic acid (**29**), which is present in the sponge,[5] as some activity (99640 dpm) was observed (see experiment 11 in Table II) in the 10-Me-16:0 acid (**6**) but no radioactivity was encountered in the 22-Me-$\Delta^{5,9}$-28:2 acid (**1**), probably because the reaction efficiency is so low that multistep reactions cannot be demonstrated when other steps are more efficient.

The order of introduction of the double bond in the triene acid **2** ($\Delta^{5,9,23}$-30:3) can also be elucidated from the incorporation experiments. We observed that no transformation occurred with palmitic acid but did so with palmitoleic acid (**29**), which already has one double bond in the correct position. Apparently, the sponge cannot introduce a double bond into the middle of the chain, but only in the C_5 and C_9 positions, which are typical of sponge LCFA's. A detailed study of the sequence of double-bond introduction by synthesis and incorporation of olefinic acids with different chain lengths and double-bond positions is currently under way in our laboratory.

Concerning the chirality of the 22-Me-$\Delta^{5,9}$-28:2 acid (**1**), no definitive conclusion could be derived from the incorporation of the pure (10*R*)- and (10*S*)-10-Me-16:0 enantiomers **26** and **27**. The lack of specificity of the elongase may be due to the fact that the asymmetric center is very far from the carboxylic acid end where the homologation takes place. Since the (10*R*)-10-Me-16:0 acid (**26**) is preferentially incorporated into the LCFA's (cf. Table II), the *R* configuration (**42**) seems to be preferred.

To elucidate the absolute configuration of the natural 22-Me-$\Delta^{5,9}$-28:2 acid (**1**), we synthesized both enantiomers starting with intermediates of known absolute configuration (Scheme VI). The first (Δ^9) cis double bond of 22-methyl-5,9-octacosadienoic acid (**1**) was generated from 1,5-cyclooctadiene **37**. Peracid oxidation[34] of **37**, after separation from the diepoxide, gave the monoepoxide **34**. Cleavage[35] of **34** with subsequent $NaBH_4$ reduction led to the diol **31**, whose monotetrahydropyranyl ether **32** was tosylated and homologated by six carbons with the Grig-

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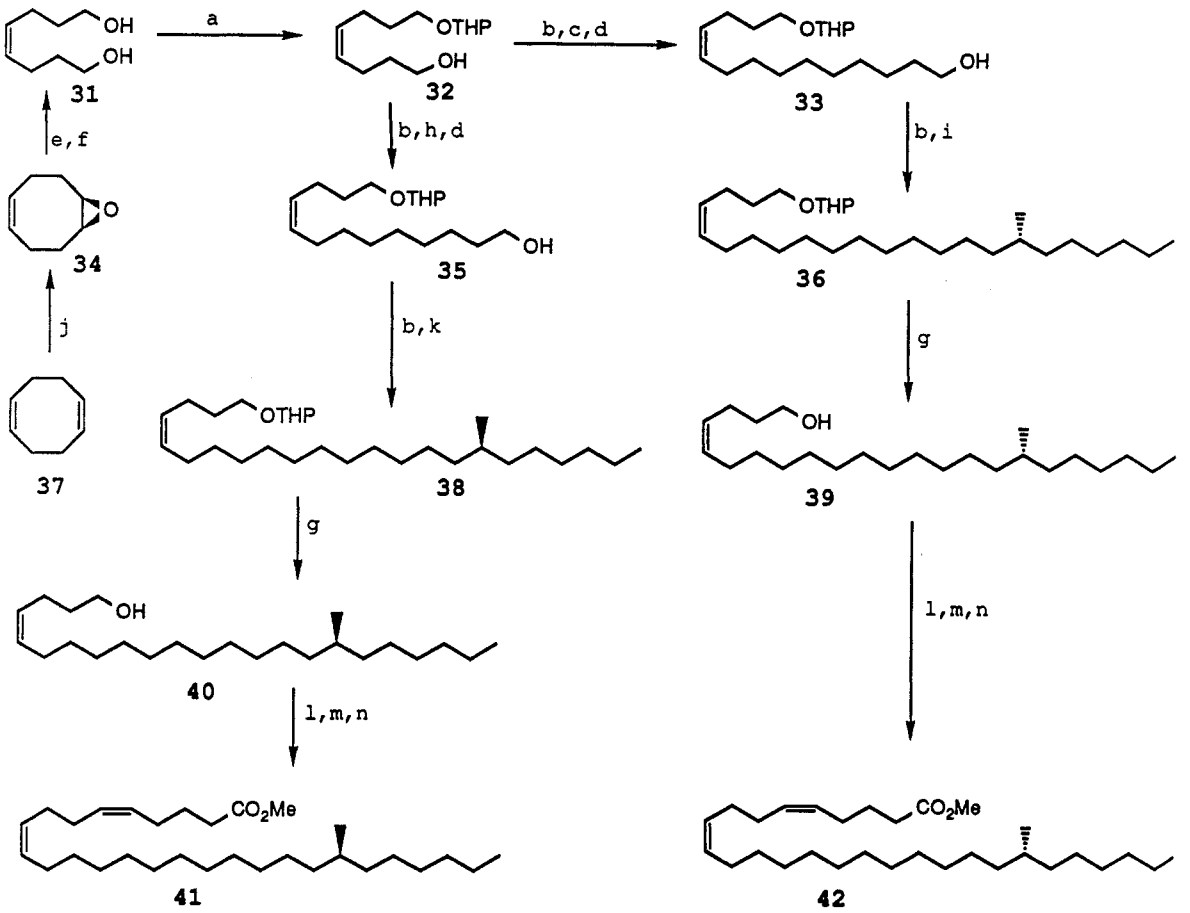
Scheme VI[a]



[a] Reagent: (a) dihydropyran, H^+, Et_2O; (b) TsCl, pyridine; (c) Li_2CuCl_4, $Me_3SiO(CH_2)_6MgCl$; (d) K_2CO_3, MeOH; (e) H_5IO_6; (f) $NaBH_4$; (g) TsOH, MeOH; (h) Li_2CuCl_4, $Me_3SiO(CH_2)_5MgCl$; (i) Li_2CuCl_4, Grignard reagent of **12**; (j) MCPBA, CH_2Cl_2; (k) Li_2CuCl_4, Grignard reagent of **17**; (l) PDC, CH_2Cl_2; (m) $Br^-Ph_3P^+(CH_2)_4CO_2H$, KH, Me_2SO, THF; (n) CH_2N_2, Et_2O.

nard reagent of 6-[(trimethylsilyl)oxy]hexyl chloride;[36] subsequent selective cleavage of the trimethylsilyl group[37] generated the alcohol **33**. In the same way a five-carbon elongation of the tosylate of **32** with the Grignard reagent of 5-[(trimethylsilyl)oxy]pentyl chloride[36] afforded the alcohol **35**. Coupling of the tosylate of **33** with the Grignard reagent of the optically active bromide **12** (Scheme II) gave the tetrahydropyranyl ether **36** with the *R* configuration at C-17.

Since the measured optical rotation of the bromide **12** (see Experimental Section) did not agree with that reported in the literature,[26] its enantiomeric purity was verified in the following manner. Displacement of the bromide with phenyl selenide anion, generated from the treatment of diphenyl diselenide with $NaBH_4$, gave the alkyl selenide, which was immediately oxidized with hydrogen peroxide with subsequent sigmatropic rearrangement to provide the terminal alkene **16**.[38] Hydroboration[39] of **16** regenerated the alcohol **19**, which was converted to the amide **11**. HPLC analysis indicated that the enantiomeric ratio remained unchanged.

Analogous coupling of the tosylate of the alcohol **35** with the Grignard reagent of the optically active bromide **17** (Scheme II) furnished the tetrahydropyranyl ether **38** with the *S* configuration. Each of the THP ethers **36** and **38** were deprotected to the *R* and *S* alcohols **39** and **40**, which had equal and opposite rotations. They were subjected to pyridinium dichromate oxidation to the aldehydes, followed by Wittig olefination with the dianionic ylide of (4-carboxybutyl)triphenylphosphonium bromide[40] and diazomethane methylation to generate the methyl esters **42** [(22*R*)-(−)-22-Me-$\Delta^{5,9}$-28:2] and **41** [(22*S*)-(+)-22-Me-$\Delta^{5,9}$-28:2], respectively. The stereoselectivity of the Wittig reaction was determined by $AgNO_3$ reverse-phase HPLC analysis to be 95% in favor of the cis-oriented Δ^5 double bond.[41] The cis,cis double-bond stereochemistry was also established by using the shift reagent[42] Yb(fod)$_3$ in $CDCl_3$.

The optical rotations obtained for the methyl esters **42** and **41** were $[\alpha]^{20}_D$ −0.17° (*c* 6.5, $CHCl_3$) and $[\alpha]^{20}_D$ +0.18° (*c* 11.9, $CHCl_3$), respectively. In view of the weak optical activity, the natural acid was reisolated in a large amount and found to display $[\alpha]^{20}_D$ −0.12° (*c* 6.7, $CHCl_3$). This result confirms that the natural 22-Me-$\Delta^{5,9}$-28:2 acid (**1**) is optically active[43] and possesses the 22*R* absolute configuration. This conclusion is consistent with earlier studies on the bacterial 10-methyloctadecanoic acid[44]

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(tuberculostearic acid) where both enantiomers were synthesized; comparison with the natural material showed it to be the (-) form with the *R* configuration.

Experimental Section

General Methods. *Aplysina fistularis* Pallas (1766) specimens were collected at Casa Cove, La Jolla, CA, from 1 to 3 m below mean lower water levels. *A. fistularis* occurs in at least two chemical forms that differ in their natural product chemistry.[45] We used the shallow form (<5 m), which contains a 9:1 mixture of aerothionin and homoaerothionin. For the methionine incorporation, specimens were transferred to Hopkins Marine Station, Pacific Grove, CA; for all other experiments, specimens were transferred to nearby Scripps Institute of Oceanography. The methionine incorporation was initiated on October 20, 1985. All other incorporations were performed in duplicate during 1986 with the sodium salt of the acids: on January 10 for the racemic mixture of 10-Me-16:0 (**6**); January 20 for palmitic acid and (10*R*)-10-Me 16:0 (**26**); March 22 for (10*S*)-10-Me-16:0 (**27**); June 25 for palmitoleic acid (**29**); and May 30 for both the (10*R*)- and (10*S*)-10-Me-16:0 acids where one specimen was cut into four portions for the duplicate experiments.

Incorporation experiments were performed according to slightly modified versions of our earlier procedures.[9,46] The ^{14}C-labeled precursors were dissolved in a small quantity of 70:30 EtOH/seawater, further diluted with seawater, and then injected directly into the sponge, which was contained in 2 L of unfiltered seawater in a 4-L glass jar with continuous aeration and maintenance at ambient ocean surface temperature (14–18 °C) with a 12-h light cycle. After 17–27 h, the seawater was discarded and a continuous flow of surface seawater was added to the jar for 7 days or for 1 month (Tables II and IV). All sponges remained healthy during this time. Finally, the sponges were frozen, lyophilized, sealed under nitrogen gas, and shipped to Stanford University for analysis.

The total lipids were then extracted by the method of Bligh and Dyer.[47] The phospholipids were separated from the neutral lipids and glycolipids by chromatography on ammonium hydroxide treated silicic acid (100–200 mesh) using the procedure of Privett et al.[48] The fatty acyl components of the neutral lipids, "glycolipids", and phospholipids were obtained as their methyl esters by reaction with methanolic hydrogen chloride followed by purification via silica gel column chromatography and elution with hexane/ether (15:1). High-performance liquid chromatography (HPLC) was performed on a Waters Associates HPLC system (M6000 pump, R403 differential refractometer or a UV detector M450 at 254 nm). For reverse-phase chromatography, Altex Ultrasphere ODS2 columns (25 cm × 10 mm i.d., two columns in series) were used; for normal phase, three Whatman Partisil M9 10/50 columns, connected in series were used. The capillary gas chromatography mass spectrometry (GC/MS) analyses were performed with a Hewlett Packard HP5995 or a HP5970 system. ^1H NMR spectra were run in $CDCl_3$ either on a Nicolet NT300WB (300 MHz), a Varian Associates XL-100 (100 MHz), or a Varian XL-400 (400 MHz) spectrophotometer. Infrared spectra were recorded on a Nicolet Model 7199 Fourier transformer spectrometer. Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. Microanalyses and high-resolution mass spectra were performed at the University of California, Berkeley.

Ozonolysis of long-chain fatty acid methyl esters were performed by dissolving them in 2 mL of BF_3/MeOH. Ozone was bubbled briskly for 2 min. The resulting solutions were transferred to capped vials (1 h, 100 °C), followed by hexane extraction and purification by silica gel column chromatography. The different fatty acid methyl esters from the cleavage were separated by HPLC. For epoxidation, the samples were dissolved in 5 mL of methylene chloride and an excess of *m*-chloroperoxybenzoic acid was added. The reaction products were directly separated by thin layer chromatography (TLC). For the radioactivity measurement small aliquots (usually 1/50 or 1/10) of the ^{14}C-labeled material were dissolved in 10 mL of organic counting scintillant (OCS), and the radioactivity was measured with a Beckman scintillation system. All results are corrected for the background radiation, calculated to correspond to the total sample, and presented as dpm by using a standard solution. Optical rotations ($[\alpha]^{20}_D$) were taken in chloroform ($CHCl_3$) or in neat liquid at the stated temperature on a Rudolph Research Autopol III polarimeter in a 1.00-dm microcell using the sodium (d) line wavelength. [1-^{14}C]Palmitic acid was obtained from ICN Radiochemicals (Irvine, CA). All other radioactive precursors were synthesized as described below.

Synthesis of Precursors. General Procedure for the Preparation of the Nitriles. The appropriate alcohol (4 mmol) was dissolved in distilled methylene chloride (20 mL) and triethylamine (1 mL), followed by methanesulfonyl chloride (0.45 mL) and catalytic amounts of 4-(dimethylamino)pyridine (DMAP, 20 mg) added dropwise at 0 °C. The reaction was stirred at room temperature for 8 h. Aqueous $NaHCO_3$ (5%) was added and the reaction mixture was extracted with CH_2Cl_2. The organic layer was dried over sodium sulfate and evaporated, and the crude mesylate was purified by column chromatography on silica gel (yield 80%). The methanesulfonate (0.34 mmol) was dissolved in 12 mL of tetrahydrofuran/dimethyl sulfoxide (1:1 v/v) and $K^{14}CN$ (1.3 mg, specific activity 57.6 mCi/mmol) and cold KCN (15 mg) were added. The reaction mixture was refluxed for 8 h under nitrogen. After the usual workup with ether (4 × 15 mL), the desired nitrile was obtained and purified by silica gel column chromatography with hexane/ether (4:1 v/v) as eluent (yield 85%).

General Procedure for the Preparation of the Acids. The radioactive nitrile was hydrolyzed by being heated under reflux for 16 h in ethanolic aqueous potassium hydroxide solution (6%), extracted with ether, and purified by silica gel column chromatography with hexane/ether (4:1 v/v). Each acid was then transformed into the sodium salt with sodium carbonate.

***dl*-9-Methyl-1-[(tetrahydropyran-2-yl)oxy]pentadecane (5).** To a stirred solution of the tetrahydropyranyl ether **4** (1.5 g), prepared from 1,8-octanediol (**3**) (from Aldrich) as described for its unsaturated analogue **32**, in 15 mL of dry pyridine was added at 0 °C 1.7 g of *p*-toluenesulfonyl chloride. After 3 h at 0 °C the usual workup with ether gave the tosylate, which was dissolved in 15 mL of THF. To this solution was added 0.2 molar equiv of premixed Li_2CuCl_4 in 1 mL of THF, followed by 2-octylmagnesium bromide (freshly prepared) in 15 mL of THF at 0 °C via a syringe. The reaction was stirred at 0 °C for 2 h and at room temperature for 2 h. Filtration through Celite after quenching with saturated NH_4Cl solution and the usual workup with ether and column chromatography on silica gel gave **5** (1.6 g, yield 75%): ^1H NMR (300 MHz, $CDCl_3$) δ 0.833 (3 H, d, J = 6.3 Hz, 9-CH_3), 0.882 (3 H, t, J = 6.6 Hz, RCH_3), 4.577 (1 H, m, OCHO); MS (70 eV), m/z (relative intensity) 326 (M^+, 0.3), 253 (1.1), 141 (3.4), 127 (6.0), 99 (9.3), 85 (100).

***dl*-9-Methylpentadecan-1-ol.** The THP ether **5** (4 g) was stirred at room temperature in MeOH (30 mL) and 0.2 g of *p*-toluenesulfonic acid for 3 h. The usual workup with ether and column chromatography on SiO_2 gave the desired alcohol (2.2 g, yield 74%): ^1H NMR (300 MHz, $CDCl_3$) δ 0.834 (3 H, d, J = 6.6 Hz, 9-CH_3), 0.882 (3 H, t, J = 6.9 Hz, RCH_3), 3.643 (2 H, t, J = 6.6 Hz, RCH_2OH); MS (70 eV), m/z (relative intensity) 224 ($M^+ - H_2O$, 0.2), 154 (1.4), 139 (4.9), 126 (3.5), 97 (16.5), 83 (50.5), 57 (100).

Methyl *dl*-9-Methylpentadecane-1-sulfonate. The mesylate was obtained following the general procedure (yield 76%): ^1H NMR (100 MHz, $CDCl_3$) δ 0.836 (3 H, d, J = 5.6 Hz, 9-CH_3), 0.881 (3 H, t, J = 7.3 Hz, RCH_3), 2.994 (3 H, s, SO_2CH_3), 4.220 (2 H, t, J = 6.7 Hz, RCH_2OSO_2Me); MS (70 eV), m/z (relative intensity) 224 (0.3), 196 (0.4), 181 (0.2), 168 (0.2), 154 (2.9), 139 (9.8), 126 (7.3), 111 (19.9), 97 (32.1), 83 (60.4), 79 (35.5), 69 (62.2), 57 (82.3), 55 (94.2), 41 (100).

***dl*-1-[^{14}C]Cyano-9-methylpentadecane** was obtained following the general procedure and further purified by reverse phase HPLC (Altex, MeOH; yield 86%); specific activity = 4.22

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mCi/mmol: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.833 (3 H, d, J = 6.0 Hz, 9-CH_3), 0.880 (3 H, t, J = 6.3 Hz, RCH_3), 2.332 (2 H, t, J = 7.2 Hz, RCH_2CN); MS (70 eV), m/z (relative intensity) 251 (M^+, 2.8), 236 (4.7), 222 (6.5), 208 (8.4), 194 (8.4), 180 (9.3), 166 (31.8), 152 (10.3), 138 (19.6), 124 (23.4), 110 (30.8), 96 (34.6), 82 (32.7), 71 (53.3), 57 (82.2), 41 (100).

***dl*-[1-^{14}C]-10-Methylhexadecanoic Acid (6).** The acid was obtained in quantitative yield from the corresponding nitrile (44 mg); specific activity = 4.37 mCi/mmol. For identification, the cold material was converted to the methyl ester with diazomethane: ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.831 (3 H, d, J = 6.3 Hz, 10-CH_3), 0.881 (3 H, t, J = 6.3 Hz, RCH_3), 2.301 (2 H, t, J = 7.5 Hz, RCH_2CO_2Me), 3.685 (3 H, s, CO_2CH_3); MS (70 eV), m/z (relative intensity) 284 (M^+, 3.2), 2.41 (2.6), 199 (3.9), 149 (3.9), 143 (16.8), 129 (6.8), 97 (7.9), 87 (34.7), 74 (56.2), 55 (50.1), 43 (100).

(3*R*)-(+)-3,7-Dimethyloct-6-enoic Acid (8, (+)-Citronellic Acid). Dry HCl gas was bubbled for 12 h through pulegone (7) (100 g, 0.66 mol, distilled from Oil of Pennyroyal, $[\alpha]^{20}_D$ +19.55° (neat, 1 dm). The mixture was poured into 300 mL of a 10% KOH aqueous solution, which was subsequently stirred at room temperature for 2 h. After being washed with ether, the aqueous layer was acidified with HCl (10 N) and extracted again with ether. The usual workup of the organic phase and distillation (90 °C, 0.1 mmHg) gave the acid 8 (45 g, yield 45%): $[\alpha]^{20}_D$ +10.2° (c 5.08, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.979 (3 H, d, J = 6.6 Hz, 3-CH_3), 1.602 and 1.683 (3 H, each, s, $C{=}C(CH_3)_2$), 5.05–5.20 (1 H, m, 6-H). Anal. Calcd for $C_{10}H_{18}O_2$: C, 70.50; H, 10.65. Found: C, 70.48; H, 10.29.

(3*R*)-(+)-3,7-Dimethyloct-6-en-1-ol (9, (+)-Citronellol). The acid 8 (10.8 g, 0.06 mol) in 50 mL of dry ether was added dropwise to a vigorously stirred suspension of LAH (8 g) in dry ether (500 mL) at 0 °C over a period of 1 h. Standard workup and distillation (65 °C, 0.5 mmHg) gave 9 (8.2 g, yield 87%): $[\alpha]^{20}_D$ +4.67° (neat, 1 dm); ^{1}H NMR (100 MHz, $CDCl_3$) δ 0.909 (3 H, d, J = 6.1 Hz, 3-CH_3), 1.600 and 1.675 (3 H, each, s, $C{=}C(CH_3)_2$), 3.680 (2 H, t, J = 6.4 Hz, CH_2OH), 5.05–5.20 (1 H, m, 6-H). Anal. Calcd for $C_{10}H_{20}O$: 76.86; H, 12.90. Found: C, 76.64; H, 13.04.

(6*S*)-(−)-2,6-Dimethyl-2-dodecene (10). To a solution of citronellol (9) (10 g, 0.06 mol) in pyridine (30 mL) was added p-toluenesulfonyl chloride (15 g) portionwise at 0 °C over a period of 1 h. The reaction was stirred for an additional 6 h at 0 °C. Excess p-toluenesulfonyl chloride was destroyed by adding lactic acid. The usual workup with ether and washing with saturated $NaHCO_3$ solution gave the tosylate, to which was added 0.2 molar equiv of Li_2CuCl_4 in dry THF (100 mL). The tosylate solution was cooled to 0 °C and was added slowly to a freshly prepared solution of 1.5 molar equiv of n-BuMgBr in THF (50 mL). The coupling reaction was worked up as described for 5 to provide the alkene 10 (9.4 g, yield 74%): bp 82 °C (2 mmHg); $[\alpha]^{20}_D$ −1.0° (c 5.42, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.852 (3 H, d, J = 6.3 Hz, 6-CH_3), 0.879 (3 H, t, J = 6.6 Hz, RCH_3), 1.602 and 1.682 (3 H, each, s, $C{=}C(CH_3)_2$), 5.05–5.20 (1 H, m, 3-H); calcd for $C_{14}H_{28}$ 196.2191, found by HMS 196.2198.

(4*S*)-(−)-4-Methyldecanol (15). Ozone was bubbled through the alkene 10 (9.4 g) in CH_2Cl_2 (200 mL) and pyridine (0.5 mL) at −78 °C until a pale blue color persisted. Excess ozone was bubbled away. $NaBH_4$ (2 g) in EtOH (20 mL) was added slowly at −78 °C, followed by stirring at 0 °C for 2 h. The usual workup with CH_2Cl_2 and chromatography on SiO_2 gave the alcohol 15 (6 g, yield 73%): bp 80 °C (0.1 mmHg); $[\alpha]^{20}_D$ −1.1° (c 5.33, $CHCl_3$); ^{1}H NMR (100 MHz, $CDCl_3$) δ 0.867 (3 H, d, J = 5.6 Hz, 4-CH_3), 0.882 (3 H, t, J = 4.3 Hz, RCH_3), 3.630 (2 H, t, J = 6.5 Hz, CH_2OH). Anal. Calcd for $C_{11}H_{24}O$: C, 76.66; H, 13.95. Found: C, 76.49; H, 13.62.

(3*R*)-3,7-Dimethyl-1-[(tetrahydropyran-2-yl)oxy]-7-octene (14). Citronellol 9 (5.8 g) in dry ether (50 mL) was refluxed with dihydropyran (7 g) and p-toluenesulfonic acid (0.5 g) for 2 h. The usual workup with ether and chromatography on SiO_2 gave 14 (8.5 g, yield 93%): bp 90–100 °C (0.1 mmHg); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.905 (3 H, d, J = 6.6 Hz, 3-CH_3), 1.537 and 1.552 (3 H each, s, $C{=}C(CH_3)_2$), 4.578 (1 H, s, OCHO), 5.05–5.20 (1 H, m, 6-H). Anal. Calcd for $C_{15}H_{28}O_2$: C, 74.93; H, 11.75. Found: C, 75.16; H, 11.87.

(4*R*)-4-Methyl-6-[(tetrahydropyran-2-yl)oxy]hexan-1-ol (21). The protected THP ether 14 (26 g) was ozonized in the same way as the alkene 10 in CH_2Cl_2 (300 mL). Reductive workup with $NaBH_4$ (4 g) in EtOH (30 mL) and chromatography on SiO_2 gave the alcohol 21 (20 g, yield 81%): bp 126–132 °C (0.5 mmHg); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.919 (3 H, d, J = 6.6 Hz, 4-CH_3), 3.657 (2 H, t, J = 6.6 Hz, CH_2OH), 4.56–4.57 (1 H, m, OCHO); MS (70 eV), m/z (relative intensity) 216 (M^+, 0.6), 132 (3.4), 116 (7.6), 102 (22.1), 98 (25.7), 86 (100). Anal. Calcd for $C_{12}H_{24}O_3$: C, 66.61; H, 11.19. Found: C, 66.80; H, 11.24.

(3*R*)-3-Methyl-1-[(tetrahydropyran-2-yl)oxy]nonane (20). The alcohol 21 (10 g) was tosylated and coupled with excess n-propylmagnesium bromide and catalyzed by Li_2CuCl_4 to give, after chromatography on SiO_2, the THP ether 20 (8.9 g, yield 79%): bp 115–125 °C (2 mmHg); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.876 (3 H, t, J = 6.6 Hz, RCH_3), 0.883 (3 H, d, J = 6.6 Hz, 3-CH_3), 4.56–4.57 (1 H, m, OCHO); MS (70 eV), m/z (relative intensity) 242 (M^+, 0.3), 142 (1.0), 112 (1.2), 102 (2.9), 86 (100). Anal. Calcd for $C_{15}H_{31}O_2$: C, 74.31; H, 12.48. Found: C, 74.42; H, 12.44.

(3*R*)-(+)-3-Methylnonan-1-ol (19). The THP ether 20 (9 g) in methanol (30 mL) was stirred at room temperature with p-toluene-sulfonic acid (0.5 g) until the starting material disappeared. The usual workup with EtOAc and column chromatography gave the alcohol 19 (5.6 g, yield 95%): bp 60–61 °C (0.1 mmHg); $[\alpha]^{35}_D$ +3.62° (neat, 1 dm) (lit.[26] $[\alpha]^{35}_D$ +0.64° (neat, 2 dm)); ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.873 (3 H, t, J = 5.7 Hz, RCH_3), 0.884 (3 H, d, J = 6.6 Hz, 3-CH_3). Anal. Calcd for $C_{10}H_{22}O$: C, 75.87; H, 14.02. Found: C, 76.02; H, 13.83.

(9*R*)-9-Methylpentadecan-1-ol (24). The alcohol 19 (3 g) was tosylated and coupled with the Grignard reagent obtained from 1-[(tetrahydropyran-2-yl)oxy]hexyl chloride (bp 105 °C at 0.5 mmHg) in the presence of catalytic Li_2CuCl_4 to give the THP ether 22 after the usual workup. Deprotection with TsOH (1 g) and MeOH (30 mL) gave the pure alcohol 24 after $SiOH_2$ column chromatography (3.4 g, yield 74%): ^{1}H NMR and MS were identical with those of the racemic compound; $[\alpha]^{20}_D$ −0.29° (c 24.28, $CHCl_3$).

(9*R*)-Methyl 9-methylpentadecane-1-sulfonate: ^{1}H NMR and MS identical with the racemic compound; $[\alpha]^{20}_D$ −0.33° (c 8.7, $CHCl_3$).

(9*R*)-1-[^{14}C]Cyano-9-methylpentadecane: ^{1}H NMR and MS identical with the racemic compound; $[\alpha]^{20}_D$ −0.28° (c 5.3, $CHCl_3$); specific activity 1.8 mCi/mmol.

(10*R*)-[1-^{14}C]-10-Methylhexacosadecanoic acid (26): ^{1}H NMR and MS identical with the racemic compound 6; $[\alpha]^{20}_D$ −0.23° (c 8.28, $CHCl_3$); specific activity 1.8 mCi/mmol.

(9*S*)-9-Methylpentadecan-1-ol (25). The alcohol 15 (3 g) was tosylated and coupled with the Grignard reagent obtained from 1-[(tetrahydropyran-2-yl)oxy]pentyl chloride (bp 103–105 °C (0.5 mmHg)) to give, after SiO_2 chromatography, the THP ether 23 (yield 74%). Deprotection of the THP ether 27 (0.9 g) gave, after chromatography, the alcohol 25 (0.6 g, yield 89%): ^{1}H NMR and MS identical with the racemic alcohol; $[\alpha]^{20}_D$ +0.33° (c 25.7, $CHCl_3$).

(9*S*)-Methyl 9-methylpentadecane-1-sulfonate: ^{1}H NMR and MS identical with the racemic compound; $[\alpha]^{20}_D$ +0.38° (c 9.3, $CHCl_3$).

(9*S*)-1-[^{14}C]Cyano-9-methylpentadecane: ^{1}H NMR and MS identical with the racemic compound; $[\alpha]^{20}_D$ +0.38° (c 9.3, $CHCl_3$); specific activity 2.6 mCi/mmol.

(10*S*)-[1-^{14}C]-10-Methylhexacosadecanoic acid (27): ^{1}H NMR and MS identical with the racemic compound 6; $[\alpha]^{20}_D$ +0.27° (c 11.8, $CHCl_3$); specific activity 3.2 mCi/mmol.

8-Pentadecen-1-ol (30). The phosphonium salt of 8-bromooctanoic acid (847 mg, 1.78 mmol) (from Aldrich) was dissolved in 10 mL of tetrahydrofuran/dimethyl sulfoxide (1:1, v/v) under nitrogen. After cooling the mixture at 0 °C, n-BuLi (1.6 N, 2 equiv) in hexane was added dropwise, resulting in the development of a deep orange-red color. The solution was stirred at room temperature for 15 min before heptanal (1.5 mmol) was added, and the reaction was then stirred at room temperature for 8 h before being poured on ice and acidified with 1 N HCl. After ether extraction the crude mixture was purified by silica gel column chromatography using hexane/ether (4:1, v/v) as eluent. The acid (165 mg) was then converted to its methyl ester with 10 mL of methanolic hydrogen chloride (1.25 N) and finally reduced to the alcohol 30 with $LiAlH_4$ (400 mg) and purified by HPLC reverse-phase chromatography (yield 37%): ^{1}H NMR (400 MHz,

$CDCl_3$) δ 0.880 (3 H, t, J = 6.2 Hz, CH_3), 3.639 (2 H, t, J = 6.72 Hz, CH_2OH), 5.345 (2 H, m, olefinic protons); MS (70 eV), m/z (relative intensity) 208 ($M^+ - H_2O$, 21.4), 152 (8.7), 138 (9.9), 124 (19.1), 109 (32), 96 (68.3), 82 (93.8), 79 (13.3), 69 (55.0), 67 (83), 55 (100), 43 (45.3), 41 (92.0).

Methyl 8-pentadecene-1-sulfonate was obtained following the general procedure: ^1H NMR (400 MHz, $CDCl_3$) δ 0.880 (3 H, t, J = 6.14 Hz, CH_3), 3.000 (3 H, s, OSO_2CH_3), 4.219 (2 H, t, J = 6.85 Hz, CH_2OMs), 5.344 (2 H, m, olefinic protons); MS (70 eV), m/z (relative intensity) 208 (15.3), 152 (3.8), 138 (5.9), 124 (12.9), 110 (23.2), 96 (56.6), 82 (73.1), 79 (38.8), 69 (46.3), 67 (81.8), 55 (100).

1-[^{14}C]Cyano-8-pentadecene was obtained by following the general procedure: ^1H NMR (400 MHz, $CDCl_3$) δ 0.883 (3 H, t, J = 7.02 Hz, CH_3), 2.335 (2 H, t, J = 7.02 Hz, CH_2CN), 5.347 (2 H, m, olefinic protons); MS (70 eV), m/z (relative intensity) 235 (M^+, 6.5), 220 (2), 206 (12.6), 192 (17.9), 178 (13.0), 164 (10.9), 150 (17.9), 136 (145.8), 122 (62.6), 108 (11.95), 97 (21.3), 94 (14.0), 83 (30.6), 81 (18.3), 69 (70), 55 (100); specific activity 2.07 mCi/mmol.

[1-^{14}C]-9-Hexadecenoic acid (29) was obtained by following the general procedure and converted to the methyl ester for identification: ^1H NMR (400 MHz, $CDCl_3$) δ 0.882 (3 H, t, J = 6.5 Hz, CH_3), 2.301 (2 H, t, J = 7.3 Hz, CH_2CO_2Me), 3.664 (3 H, s, CO_2CH_3), 5.340 (2 H, m, olefinic protons); MS (70 eV), m/z (relative intensity) 268 (M^+, 3), 236 (12.1), 194 (10.2), 152 (11.7), 111 (15.2), 96 (31.6), 87 (37.3), 83 (35.8), 69 (52.2), 55 (100); specific activity 2.12 mCi/mmol.

(4*S*)-(+)-1-Bromo-4-methyldecane (17). The alcohol **15** (17 g) was tosylated with p-toluenesulfonyl chloride (25 g) in pyridine (100 mL). The tosylate, isolated after the usual workup with ether, was dissolved in dry acetone (200 mL) and LiBr (24 g) was added in one portion to the tosylate solution, which was refluxed overnight. The usual workup with pentane, filtration through SiO_2, and distillation (bp 78 °C (0.1 mmHg)) gave the bromide **17** (20 g, yield 86%): $[\alpha]^{20}_D$ +2.7° (c 5.10, $CHCl_3$); ^1H NMR (100 MHz, $CDCl_3$) δ 0.868 (3 H, d, J = 5.6 Hz, 4-CH_3), 0.884 (3 H, t, J = 4.4 Hz, RCH_3), 3.390 (2 H, t, J = 6.8 Hz, CH_2Br). Anal. Calcd for $C_{11}H_{23}Br$: C, 56.15; H, 9.86. Found: C, 56.21; H, 9.84.

(3*R*)-(−)-1-Bromo-3-methylnonane (12). The alcohol **19** (10 g) was tosylated and displaced with LiBr in acetone, as previously described, to give, after chromatography on SiO_2 and distillation (70–72 °C, 0.1 mmHg), the bromide **12** (11.7 g, yield 84%): $[\alpha]^{20}_D$ −6.85° (neat, 1 dm), $[\alpha]^{27}_D$ −6.55° (neat, 1 dm), lit.[26] $[\alpha]^{27}_D$ −2.48° (neat, 2 dm); ^1H NMR (300 MHz, $CDCl_3$) δ 0.882 (3 H, t, J = 6.9 Hz, RCH_3), 0.882 (3 H, d, J = 6.6 Hz, 3-CH_3), 3.41–3.45 (2 H, m, CH_2Br). Anal. Calcd for $C_{10}H_{21}Br$: C, 54.28; H, 9.57. Found: C, 54.39; H, 9.49.

(*R*)-3-Methylnon-1-ene (16). The bromide **12** (4 g) was added to a solution of diphenyl diselenide (6 g) in EtOH (50 mL), which was premixed with $NaBH_4$ (2 g) until no more hydrogen bubbled out, and refluxed for 4 h. After cooling to 0 °C, H_2O_2 (30%, 20 mL) was added and heated at 60 °C for 3 h. The workup with pentane and distillation (80 °C to 120 °C at atmospheric pressure) gave the terminal alkene **16** (1.5 g, yield 59%): ^1H NMR (300 MHz, $CDCl_3$) δ 0.878 (3 H, t, J = 6.9 Hz, RCH_3), 0.974 (3 H, d, J = 6.6 Hz, 3-CH_3), 4.87–4.98 (2 H, m, C=CH_2) 5.60–5.73 (1 H, m, 2-H); MS (70 eV), m/z (relative intensity) 140 (1.1), 111 (19.3), 97 (3.3), 83 (17.1), 70 (100).

A 1.2-g sample of the alkene **16** in dry THF (10 mL) was added via a syringe to a preheated solution of 9-BBN (9 mL of a 0.5 N THF solution). The reaction was refluxed for 1 h and cooled to 0 °C, and EtOH (5 mL) with NaOH (6 N, 2 mL) and H_2O_2 (30%, 5 mL) were added and heated at 50 °C for 1 h. Workup with EtOAc and chromatography oon SiO_2 gave the alcohol **19** (0.9 g, 66%). The ^1H NMR spectrum was identical with that of the original alcohol **19**. This alcohol (prepared from the alkene **16**) was oxidized and converted to the amide **11**, which, upon HPLC analysis, showed the enantiomeric ratio to have remained unchanged (97:3).

Preparation of Amide Derivatives 11 and 13. The appropriate acids **8** (220 mg) and **18** were refluxed with oxalyl chloride (0.2 mL) in benzene (10 mL) for 1 h, after which the solvent was evaporated. The acid chloride was dissolved in dry ether (10 mL) at 0 °C, (*S*)-(−)-α-methylbenzylamine was added dropwise, and the mixture was stirred at room temperature overnight. The usual workup with ether and column chromatography gave the amides (yield 86%), which were analyzed for their diastereoisomeric ratios by normal-phase HPLC with hexane/EtOAc (7:3, v/v) as eluent.

13: mp 68 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 0.938 (3 H, d, J = 6.5 Hz, 3-CH_3), 1.494 (3 H, d, J = 7 Hz, CH_3CHPh), 1.580, 1.669 (3 H, each, s, C=$C(CH_3)_2$), 5.061 (1 H, t, J = 6Hz, CH=C), 5.157 (1 H, m, R_2CHNH), 5.603 (1 H, d, J = 8 Hz, NH); MS (70 eV), m/z (relative intensity) 273 (M^+, 39.8), 190 (18.3), 163 (38.8), 120 (40.8), 105 (100), 86 (10.8), 79 (13.7), 77 (16.2), 69 (20.5), 59 (12.8), 41 (25.7); $[\alpha]^{20}_D$ −70.5° (c 6.22, $CHCl_3$).

11: mp 53 °C; ^1H NMR (400 MHz, $CDCl_3$) δ 0.870 (3 H, t, J = 6.9 Hz, CH_3), 0.920 (3 H, d, J = 6.4 Hz, 3-CH_3), 1.495 (3 H, d, J = 7.1 Hz, CH_3CHPh), 5.158 (1 H, m, R_2NH), 5.607 (1 H, d, J = 6.8 Hz, NH); MS (70 eV), m/z (relative intensity) 275 (M^+, 16.6), 190 (15.7), 163 (100), 120 (30.3), 105 (81.5), 79 (10.4), 77 (10.9), 69 (5.2), 59 (12), 41 (15.1); $[\alpha]^{20}_D$ −71.6° (c 5.95, $CHCl_3$).

***(Z)-cis*-1,2-Epoxy-5-cyclooctene (34).** To a solution of (*Z*,*Z*)-1,5-cyclooctadiene (**37**) (65 g, 0.60 mol) (Aldrich Chem. Co.) in CH_2Cl_2 (800 mL) was added slowly, at 0 °C, m-chloroperoxybenzoic acid (121 g, 85% by weight, 0.60 mol) in CH_2Cl_2 (500 mL). The suspension was stirred at 0 °C for 2 h and at room temperature for 1 h. Filtration, evaporation of the solvent, separation on SiO_2, and distillation (bp 55 °C at 1 mmHg) gave the monoepoxide **34** (30 g, yield 40%): ^1H NMR (100 MHz, $CDCl_3$) δ 3.00–3.08 (2 H, m, 1-H and 2-H), 5.50–5.61 (2 H, m, olefinic protons). Anal. Calcd for $C_8H_{12}O$: C, 77.36; H, 9.75. Found: C, 77.19; H, 9.56.

(*Z*)-Oct-4-ene-1,8-diol (31). To a solution of monoepoxide **34** (10 g, 80.6 mmol) in p-dioxane (40 mL) at 0 °C was added slowly periodic acid (20 g, 88 mmol) in water (40 mL). The reaction was stirred at 0 °C for 2 h and then extracted with ether. The ether solution was concentrated to 200 mL and to it was added $NaBH_4$ (5 g) in EtOH (40 mL) slowly at 0 °C. The suspension was stirred 3 h at 0 °C and 1 h at room temperature. Excess $NaBH_4$ was destroyed by slow, dropwise addition of HCl (5 N) solution at 0 °C. The mixture was extracted with EtOAc. Evaporation of the solvent and chromatography on SiO_2 gave the diol **31** (yield 64%): ^1H NMR (100 MHz, $CDCl_3$) δ 1.56–1.77 (4 H, m, 2 × CH_2CH_2OH), 2.09–2.28 (4 H, t, 3-CH_2 and 6-CH_2), 3.650 (4 H, t, J = 6.1 Hz, 2 × CH_2OH), 5.36–5.46 (2 H, m, olefinic protons); MS (70 eV), m/z (relative intensity) 144 (M^+, 2.2), 126 (4.9), 114 (5.7), 108 (6.0), 98 (19.8), 95 (26.6), 93 (67.7), 81 (56.4), 67 (100), 55 (75); calcd for $C_8H_{16}O_2$ 114.1150, found: 144.1150 (by HMS).

(*Z*)-8-[(Tetrahydropyran-2-yl)oxy]oct-4-enol (32). To a refluxing solution of the diol **31** (10 g, 69 mmol) in 100 mL of anhydrous ether and p-toluenesulfonic acid (0.5 g) was added dropwise dihydropyran (6 g, 71 mmol) in ether (20 mL). The reaction was refluxed for 5 h. The usual workup and column chromatography gave, in order of elution, the di-THP ether (4 g, 19%), mono-THP ether **32** (7.7 g, 49%), and starting diol **31** (3 g, 30%). The mono-THP ether **32** decomposed upon vacuum distillation: ^1H NMR (100 MHz, $CDCl_3$) δ 2.05–2.18 (4 H, m, 3-CH_2 and 6-CH_2), 4.56 (1 H, s, OCHO), 5.35–5.46 (2 H, m, olefinic protons); MS calcd for $C_{13}H_{24}O_3$ 228.1725, found 228.1726 (by HMS).

(*Z*)-14-[(Tetrahydropyran-2-yl)oxy]-10-tetradecen-1-ol (33). The tosylate prepared from the mono-THP ether alcohol **32** (4 g, 17.5 mmol) was dissolved in THF (30 mL) with 0.2 molar equiv of premixed Li_2CuCl_4 at 0 °C. To this solution was added, via syringe, freshly prepared Grignard reagent of 6-[(trimethylsilyl)oxy]hexyl chloride. The reaction was kept at 0 °C for 2 h and at room temperature for 2 h. After quenching with saturated NH_4Cl and the usual workup with ether (after filtration through Celite), flash chromatography was performed to remove polar impurities. The nonpolar mixture was stirred in a K_2CO_3/MeOH solution for 30 min at room temperature. Filtration, the usual workup with ether, and column chromatography gave the elongated alcohol **33** (4.6 g, yield 88%): ^1H NMR (300 MHz, $CDCl_3$) δ 2.00–2.20 (4 H, m, 9-CH_2 and 12-CH_2), 4.55–4.58 (1 H, m, OCHO), 5.34–5.39 (2 H, m, olefinic protons); MS (70 eV), m/z (relative intensity) 210 ($M^+ - 102$, 1.0), 121 (1.2), 101 (3.6), 95 (4.5), 85 (100).

(*Z*)-13-[(Tetrahydropyran-2-yl)oxy]-9-tridecen-1-ol (35). The same procedure as for the alcohol **33** was employed to convert the THP ether **32** (1.8 g, 7.89 mmol) with an excess of the Grignard

reagent of 5-[(trimethylsilyl)oxy]pentyl chloride to the alcohol **35** (2.1 g, yield 89%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.00–2.20 (4 H, m, 8-CH_2 and 11-CH_2), 4.562 (1 H, s, OCHO), 5.34–5.45 (2 H, m, olefinic protons); MS, m/z 298 (M^+), 280, 257, 215, 196.

(*R*)-17-Methyl-1-[(tetrahydropyran-2-yl)oxy]-4(*Z*)-tricosene (36). The THP ether alcohol **33** (1 g) was tosylated and reacted with the Grignard reagent of the bromide **12** (9 g) in the usual manner to give the THP ether **36** after SiO_2 chromatography (1.29 g, yield 86%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 0.830 (3 H, d, J = 6.3 Hz, RCH_3), 0.877 (3 H, t, J = 6.6 Hz, 17-CH_3), 4.573 (1 H, s, OCHO), 5.30–5.45 (2 H, m, olefinic protons); MS (70 eV), m/z (relative intensity) 334 (M^+ – 102, 1.9), 124 (2.2), 111 (3.3), 97 (4.8), 85 (100).

(*S*)-17-Methyl-1-[(tetrahydropyran-2-yl)oxy]-4(*Z*)-tricosene (38). The THP ether alcohol **35** (1 g) was tosylated and coupled with the Grignard reagent prepared from the bromide **17** (6 equiv) with Li_2CuCl_4 to give the THP ether **35** after column chromatography (1.24 g, yield 84%): ^{1}H NMR, MS identical with the enantiomer compound **36**.

(*R*)-(−)-17-Methyl-4(*Z*)-tricosen-1-ol (39). The THP ether **36** (2.2 g) was stirred at room temperature for 2 h with MeOH (30 mL) and *p*-toluenesulfonic acid (0.2 g). The usual workup and column chromatography gave the alcohol **39** (1.76 g, nearby quantitative yield): ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.832 (3 H, d, J = 6.3 Hz, 17-CH_3), 0.879 (3 H, t, J = 6.7 Hz, RCH_3), 1.632 (1 H, quintuplet, J = 6.7 Hz, 2-CH_2), 2.031 (2 H, q, J = 6.4 Hz, 6-CH_2), 2.125 (2 H, q, J = 6.5 Hz, 3-CH_2), 3.661 (2 H, t, J = 6.6 Hz, CH_2OH), 5.34–5.45 (2 H, m, olefinic protons); MS (70 eV), m/z (relative intensity) 352 (M^+, 10.8), 334 (17.0), 264 (11.4), 137 (22.4), 124 (34.6), 110 (35.4), 96 (58.0), 82 (93.8), 71 (66.5), 68 (83.8), 57 (100), 55 (86.8), 43 (98.1); $[\alpha]^{20}_D$ −0.2° (*c* 5.58, $CHCl_3$).

(*S*)-(+)-17-Methyl-4(*Z*)-tricosen-1-ol (40) was obtained as described for its enantiomer **39**: ^{1}H NMR and MS identical with the enantiomer compound **39**; $[\alpha]^{20}_D$ +0.2° (*c* 5.99, $CHCl_3$).

(22*S*)-Methyl 22-Methyl-5(*Z*),9(*Z*)-octacosadienoate (41). The alcohol **40** (800 mg) was stirred with pyridinium dichromate (3 g) in CH_2Cl_2 (25 mL) for 8 h. Filtration through Florisil and flash column chromatography gave the aldehyde (760 mg), which was dissolved in a small amount of THF and added to a solution of the Wittig dianionic salt, prepared by addition of (4-carboxybutyl)triphenylphosphine bromide (3 g) in Me_2SO to potassium hydride (550 mg) in Me_2SO, under argon with subsequent stirring for 1 h. After 4 h at room temperature, the mixture was hydrolyzed with ice–water, acidified with 30% H_3PO_4, and extracted with hexane. Column chromatography gave the acid (276 mg, yield 35%), which was converted to the methyl ester **41** with diazomethane. The cis selectivity (>95%) was verified by reverse-phase HPLC using $AgNO_3$ (50 mM) in MeOH–H_2O (95:5, v/v) as solvent: IR (film) 3007 (CH=CH), 1740 (C=O), 721 (CH=CH, cis); ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.830 (3 H, d, J = 6.4 Hz, 22-CH_3), 0.879 (3 H, t, J = 6.8 Hz, CH_3), 1.255 (30 H, s, aliphatic CH_2), 1.688 (2 H, quintuplet, J = 7.2 Hz, 3-CH_2), 1.98–2.50 (8 H, m, 4-, 7-, 8-, 11-CH_2), 2.313 (2 H, t, J = 7.4 Hz, 2-CH_2), 3.665 (3 H, s, OCH_3), 5.34–5.45 (4 H, br m, olefinic protons); MS (70 eV), m/z (relative intensity) 448 (M^+, 10.8), 416 (4.1), 306 (4.3), 182 (8.6), 168 (7.6), 164 (6.7), 150 (22.2), 141 (31.1), 136 (17.8), 125 (10.8), 123 (17.7), 109 (40.7), 97 (38.9), 81 (100), 67 (70.8), 57 (97.2); $[\alpha]^{20}_D$ +0.18° (*c* 11.9, $CHCl_3$).

(22*R*)-Methyl 22-methyl-5(*Z*),9(*Z*)-octacosadienoate (42) was obtained by following the same procedure as for **41**: ^{1}H NMR and MS of **42** were identical with those of **41**; $[\alpha]^{20}_D$ −0.17° (*c* 31.7, $CHCl_3$). This material was also identical with a sample isolated from *A. fistularis*.

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A Route for the Construction of the Taxane BC Substructure

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A stereospecific synthesis of taxane BC intermediate **6** from photoproduct **17** is described. Vinylogous imides **12–14** were prepared in four steps from cyclohexanone cyanohydrin and dimedone. Their irradiation led to respective photoproducts **15–21**. Unequivocal identification of new photoproduct structural types exemplified by **18** and **20** was made through their X-ray crystallographic analyses. Photoproduct **17** then provided **41**, which underwent fragmentation to deliver **42**. Hydrolysis of **42** and subsequent formylation led to **43**, which then furnished **6**. An alternative sequence provided epimer **30** from photoproduct **15**. The stereochemistries and thermodynamic stereochemical preferences for the ring fusions in various intermediates as well as the **6**/**30** epimeric pair were determined through a combination of NOE difference spectroscopy and chemical interconversions. The preparation of **6** models a potentially general approach to taxane BC ring construction.

Possessing an unusual highly oxygenated and stereochemically rich diterpenoid skeleton replete with bridgehead olefin in the A ring, medium B ring, and rare 3-oxygenated oxetane fused to the C ring, taxol[3] (**1**), is recognized currently as a challenging total synthesis target of some significance. Its substantial antileukemic and antitumor activities have led to the clinical testing of taxol as an anticancer chemotherapeutic agent both in this country and in Europe. The demonstration beginning in

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